IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:	Allen Comer *et al.*		
Serial No.:	10/087,346	Group No.:	1632
Filed:	03/01/02	Examiner:	Chen
Entitled:	**Skin Substitutes with Improved Barrier Function**		

DECLARATION OF DR. ALLEN COMER PURSUANT TO §1.132

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

CERTIFICATE OF MAILING UNDER 37 C.F.R. § 1.8(a)(1)(i)(A)

I hereby certify that this correspondence (along with any referred to as being attached or enclosed) is, on the date shown below, being deposited with the U.S. Postal Service with sufficient postage as first class mail in an envelope addressed to: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.

Date: 12-19-03 ~~12/18/03~~ By: Mary Ellen Warke

I, Dr. Allen Comer, state as follows:

1. My present position is Assay Development Manager, Stratatech Corporation.

2. I am an inventor of the above referenced patent application.

3. I have reviewed the Office Action dated September 25, 2003 and the references cited therein. It is my understanding that the Examiner states that the application is not enabling for a human skin equivalent having a surface electrical capacitance (SEC) of 40-240 pF or 80 - 120 pF in vivo or in vitro. The experimental evidence described below establishes that the methods described in the application are suitable for the production of skin equivalents with the specified SEC properties.

4. Skin equivalents of the STRATAGRAFT format were produced essentially as described in Example 5 of the Experimental section of the application with the exception that Clonetics FGM or equivalent medium was utilized for the initial expansion of fibroblasts. Briefly, frozen

NHDF cells are thawed and plated in Clonetics FGM medium. The cells were fed FGM medium or equivalent medium the next day to remove residual cryoprotectant and subsequently to maintain cell growth. Preconfluent NHDF cells were harvested for use in the dermal equivalent. To prepare the dermal equivalent, rat tail tendon collagen (Type I, Becton-Dickinson) was first diluted to 3 mg/ml in 0.03N acetic acid and chilled on ice. A mixture of concentrated Ham's F12 medium (8.7X normal strength, buffered with HEPES at pH 7.5) was mixed with Fetal Clone II. These two solutions were approximately 11.5 and 10% of the final solution volume. 1 N NaOH was added to the medium mixture (2.5% of final solution). The diluted collagen (74%) was then added to the mixture. A 2% volume of suspended fibroblasts (2.5 x 10^6 cells/ml for the dermal equivalent of STRATATEST and 1 x 10^6 cells/ml for dermal equivalent of STRATAGRAFT) was added to the mixture. The solution was mixed gently but thoroughly. The STRATAGRAFT skin equivalent uses TRANSWELL inserts from Corning. A 13 ml dermal equivalent was poured into each insert. For STRATAGRAFT dermal equivalents, 80 ml of FM was placed around the TRANSWELL insert in a 150 mm tissue culture dish and 10 ml was placed on top of the dermal equivalent. The inserts were placed in 37°C, 5% CO_2, 90% relative humidity incubator until used. One day prior to seeding the dermal equivalents with NIKS cells, they were lifted to the air interface by placing them onto a sterile stainless steel mesh with two wicking pads (S&S Biopath) on top to supply medium through the bottom of the tissue culture insert.

For NIKS growth and seeding, feeders were prepared fresh or thawed and plated in TM one day prior to NIKS plating. NIKS cells were plated onto the feeders at a density of approximately 3 x 10^5 cells per 100 mm dish. If the NIKS cells were newly thawed, they were fed fresh NM one day post-plating to remove residual cryoprotectant. The NIKS cells were fed NM to maintain growth as required. When the cells approached confluence, the NIKS cells were harvested, counted, and resuspended in PM. Approximately 4.65 x 10^5 NIKS cells/cm^2 were seeded onto the surface of the TRANSWELL dermal equivalents, which had been lifted to the air interface for one day. The dishes were fed PM to flood underneath the metal lifter and placed back into the incubator. Two days later, the cultures were fed SMA. After an additional two days, the cultures were fed SMB and transferred to a 75% humidity incubator where they remained, maintained with additional SMB feedings, until mature.

5. Surface electrical capacitance (SEC) measurements were obtained from four independent batches of skin tissue prepared as described in Paragraph 4. Four independent SEC measurements were taken from different areas of each tissue, and the average change in capacitance during the 10 sec measurement period was determined for each tissue. (See Example 1, p. 44 of the application, for a description of SEC readings). Data from this analysis is presented in Table 1. Three of the batches had changes in capacitance that were between 40 and 240 pF, while two of the batches had vales that were between 80 and 120 pF. On average, the four batches exhibited SEC values of 208 pF. These results demonstrate that the methods described in the application allow the production of skin tissue with SEC values within the ranges in the claims.

Table 1- SEC measurements of cultured skin tissue

	Reading	Initial	10 sec	ΔpF	Avg ΔpF
SG-032902.02	1	17.53436	250.7396	233.2052	
	2	4.025405	60.17055	56.14514	
	3	4.025405	70.51397	66.48857	
	4	15.31292	91.17404	75.86112	
					107.925
SG-112001.02	1	15.31292	93.24988	77.93696	
	2	45.55086	338.8604	293.3096	
	3	6.308294	131.5857	125.2774	
	4	32.79661	107.8984	75.10181	
					142.9064
SG-111301.02	1	84.96288	472.1913	387.2285	
	2	39.20551	412.3928	373.1873	
	3	30.64431	107.8984	77.25411	
	4	2205.51	3249.197	1043.687	
					470.3392
SG-110601.02	1	21.94439	97.41216	75.46777	
	2	26.3133	87.03094	60.71764	
	3	49.75115	266.7056	216.9545	
	4	1.72902	91.17404	89.44502	
					110.6462
			Avg of 4 batches		207.9542

6. I further declare that all statement made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are

punishable by fine or imprisonment, or both, under section 1001 of title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Allen R Comer

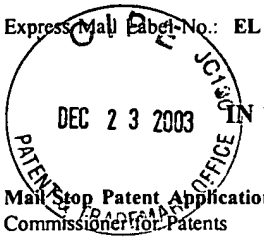
Dr. Allen Comer

Date: 12-18-03

PATENT

Express Mail Label No.: **EL 992 783 765 US**

Attorney Docket No.: **FORS-08497**



IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Mail Stop Patent Application
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

NEW APPLICATION TRANSMITTAL

Transmitted herewith for filing is the patent application of James E. Dahlberg, Hatim T. Allawi, Victor Lyamichev, Bruce P. Neri, Marilyn Olson-Munoz, LuAnne Chehak, and Sarah M. Olson for **Detection of Small Nucleic Acids**.

CERTIFICATION UNDER 37 C.F.R. § 1.10

I hereby certify that this New Application Transmittal and the documents referred to as enclosed therein are being deposited with the U.S. Postal Service on this date, **December 18, 2003**, in an envelope as "Express Mail Post Office to Addressee" Mailing Label Number **EL 992 783 765 US**, addressed to: **Mail Stop Patent Application,** Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.

Susan M. McClintock

Susan M. McClintock

1. **Type Of Application**

This new application is for a(n)

☒ Original (nonprovisional)

2. **Papers Enclosed That Are Required For Filing Date Under 37 C.F.R. § 1.53(b) (Regular) or 37 C.F.R. § 1.153 (Design) Application**

65 Pages of Specification
4 Pages of Claims
1 Page of Abstract
27 Sheets of Informal Drawings

3. **Declaration**

☒ Enclosed

☒ Unexecuted.

4. **Inventorship Statement**

The inventorship for all the claims in this application is:

☒ the same

5. **Language**

☒ English

6. **Fee Calculation (37 C.F.R. § 1.16)**

☒ Regular application

CLAIMS AS FILED

Number Filed	Number Extra	Rate	Basic Fee - $770.00 (37 C.F.R. § 1.16(a))
Total Claims (37 C.F.R. § 1.16(c))	31 - 20 =	11 × $18.00 =	$198.00
Independent Claims (37 C.F.R. § 1.16(b))	2 - 3 =	0 × $86.00 =	$0.00
Multiple Dependent Claim(s), if any (37 C.F.R. § 1.16(d))		+ $290.00 =	$0.00
		Filing Fee Calculation	$968.00

7. **Small Entity Statement(s)**

☒ Verified Statement(s) that this is a filing by a small entity under 37 C.F.R. §§ 1.9 and 1.27.

Filing Fee Calculation (50% of above) $484.00

8. Fee Payment Being Made At This Time

☒ Enclosed

☒ basic filing fee $484.00

Total Fees Enclosed **$484.00**

9. Method of Payment of Fees

☒ Check in the amount of **$484.00**

10. Authorization To Charge Additional Fees and Credit Overpayment

☒ The Commissioner is hereby authorized to charge payment of any fees associated with this communication, and/or credit any overpayment, to Deposit Account No.: **08-1290**. **An originally executed duplicate of this transmittal is enclosed for this purpose.**

11. Power of Attorney by Assignee

☒ Enclosed (unexecuted)

12. Return Receipt Postcard

☒ Enclosed

Dated: December 18, 2003

Mary Ann D. Brow
Registration No.: 42,363

MEDLEN & CARROLL, LLP
101 Howard Street, Suite 350
San Francisco, California 94105
608/218-6900

☒ **Statement Where No Further Pages Added**

☒ This transmittal ends with this page.